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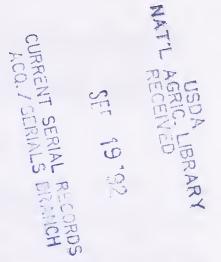
Research Paper RM-310



# Evaluating the Microscopic Fecal Technique for Estimating Hard Mast in Turkey Diets

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# **Evaluating the Microscopic Fecal Technique** for Estimating Hard Mast in Turkey Diets

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#### **Abstract**

Wild and domestic dark turkeys (*Meleagris gallopavo*) were fed experimental diets containing acorn (*Quercus gambelli*), ponderosa pine (*Pinus ponderosa*) seed, grasses, forbs, and arthropods. In fecal estimates of diet composition, acorn and ponderosa pine seed were underestimated and grass was overestimated. Regression of acorn and pine seed in experimental diets with microscopic fecal estimates indicated significant nonlinear relationships. Based on regression analyses, corrected acorn and pine seed composition in diets did not differ from experimental diets. Corrected estimates for grass were slightly overestimated. Corrected estimates for forbs and arthropods were slightly underestimated. Corrected diet estimates had smaller standard errors than microscopic fecal estimates.

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## **Evaluating the Microscopic Fecal Technique for Estimating Hard Mast in Turkey Diets**

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#### Introduction

Microscopic fecal analysis (Sparks and Malachek 1968) has been used extensively to estimate diet composition of herbivorous animals. The accuracy of this technique for estimating diet composition may be confounded by differential digestibility of plant species and fragmentation of woody plant materials (Slater and Jones 1971; Holechek and Valdez 1985a, 1985b). Other authors maintain that the technique is useful for estimating diets of herbivorous animals (Anthony and Smith 1974; Johnson and Pearson 1981; Alipayo et al. 1992). Microscopic fecal analysis produced good qualitative and quantitative estimates of diet composition for black grouse (Lyrurus tetrix) (Marti 1982). Doerr and Guthery (1983) reported that microscopic analysis of feces generally reflected composition and seasonal trends in diets of lesser prairie chickens (Tympanuchus pallidinctes).

Three assumptions are necessary in the application of the microscopic technique for estimating diets from fecal material: (1) microfragments of plant taxa are randomly distributed within the sample; (2) microfragments of plant taxa are the same size; and (3) dry weight bulk densities of all plant taxa are the same (Johnson 1982). The first two assumptions are generally accepted (Havstad and Donart 1978; Johnson 1982). The third assumption may not be true; weight per unit area of plant epidermis varies among species and stages of maturity (Heady and Van Dyne 1965). Because vegetative parts of plants have a relatively high surface area to dry weight (or volume) ratio and there are no known bacteria that digest cutin of epidermal plant cells (Johnson et al. 1983a), any bias associated with diet estimates of vegetative plant parts made from the microscopic fecal technique should be small (Johnson et al. 1983b). For mast items, characteristic trichomes used for identification of diet components are found only in the hulls (Johnson et al. 1983a). The relative ratio between unidentifiable pulp and hulls of mast is large and violation of the third assumption could result in biased estimates of diet composition.

The objectives of this study were to (1) compare estimates of diets containing mast items made from microscopic fecal analysis with known diet composition, and (2) develop corrections for bias of mast item estimates in diets due to violating the assumption that all plant taxa has the same dry weight bulk density.

#### **Methods**

This study was conducted over a period of 5 years. We conducted experiments using 4 turkeys at a time, including 4 Rio Grande wild turkeys (Meleagris galloparo intermedia) obtained from a licensed game farm and 8 dark-colored domestic turkeys. Turkeys were placed in wire cages for 2 weeks prior to the experiments and fed ad libitum a diet of commercial wild bird seed mix, commercial chicken feed, acorns (Quercus gambelli), and ponderosa pine (Pinus ponderosa) seed.

We formulated 15 experimental diets consisting of acorn, ponderosa pine seed, grass, forbs, and grasshoppers (Family Acrididae) or crickets (Family Gryillidae). Acorn, ponderosa pine seed, grasses, and arthropods are common in the diets of Merriam's turkeys in the Black Hills (Petersen and Richardson 1975; Rumble 1990). Alfalfa (*Medicago sativa*) was included to represent forbs in the diet. We ground experimental diet materials to pass a 4-mm mesh screen in a Wiley mill. We developed diet proportions based on weight and thoroughly mixed them with vegetable oil to prevent separation and ensure diets were consumed in the same proportion as presented.<sup>3</sup>

We withheld all food from our experimental birds for at at least 8 hours prior to feeding them the experimental diets. After experimental diets had been presented for 24 hours, we removed all fecal material in the cages. We collected fecal samples resulting from experimental diets over the next 8-24 hours. Samples from individual turkeys were kept separate. Fecal samples were oven-dried and sent to the Diet Composition Laboratory at Colorado State University for analyses. Samples were analyzed by reading 100 fields (20 each from 5 slides).

We applied paired *t*-tests to the hypothesis that microscopic fecal diet estimates did not differ from

<sup>3</sup> Dr. E. Keinholz, retired professor, Poultry Science, Colorado State University, personal communication.

<sup>4</sup>Use of trade, firm, or corporation names in this publication does not constitute an official endorsement or approval by the U.S. Department of Agriculture to the exclusion of others which may be suitable.16 U.S.C. 1600-1614.

experimental diets. Nonlinear regression analysis (SPSS 1990) was used to estimate relationships between microscopic fecal estimates (dependant variable) and known composition of mast items (independent variable). Additional data representing the theoretical limit of 100% experimental and fecal estimates of acorn and pine seed in diets were added to the data and weighted in regression analyses by a factor of 4 (number of turkeys fed experimental diets). Corrected estimates of acorn and pine seed in diets were based on inverse predictions from the regression analyses (Dapson 1980). We used the following formula to reconcile composition of grass, forbs, and arthropods (nonmast items) so total composition equaled 100% in corrected diets:

$$X_i = \{1-[Acorn + Pine (corrected)] / 100\} * (O_i / \sum_{i=1}^{n} O_i)$$

where

 $X_i$  = adjusted value of nonmast items

O<sub>i</sub> = observed values for nonmast items.

We then averaged corrected estimates of diets for birds of each experimental diet and applied paired *t*-tests to the hypothesis that corrected diets did not differ from experimental diets. We evaluated the accuracy of fecal and corrected diet estimates for experimental diets. This was done by comparing standard errors of the differences between these diet estimates with known composition of diets (Reese 1960).

#### **Results**

Microscopic fecal estimates of acorn, pine seed, and grass differed from composition of these items in experimental diets (table 1). Both acorn and pine seed were significantly underestimated ( $P \le 0.002$ ) by the micro-

scopic fecal technique. Consequently, composition of grass, forbs, and arthropods in turkey diets were overestimated by the microscopic fecal technique; only estimates of grass in diets differed significantly ( $P \le 0.001$ ) from composition in experimental diets.

Acorn and pine seed from microscopic fecal estimates are predictable from nonlinear regression analyses (fig. 1 and 2). Acorn composition in fecal estimates was predicted by AFECAL=  $0.012 * ADIET^{1.943}$  ( $R^2 = 0.64, P < 0.01$ ). Pine seed composition in fecal estimates was predicted by PFECAL =  $0.087 * PFECAL^{1.536}$  ( $R^2 = 0.82, P < 0.001$ ).  $R^2$  values are based on analyses without the theoretical limits of 100 included. Bias associated with microscopic fecal estimates of these mast items in turkey diets was greater when acorn and pine seed comprised a smaller portion of the diet. Higher variability in the regression equation for acorn may have resulted from high incidence of *Curcurlio* spp. larvae parasitism (Furniss and Carolin 1977) in the acorns.

Reverse predictions of diet composition, based on the nonlinear relationships expressed above, resulted in reduction of the bias from fecal estimates for 3 of 5 food items in the diets. Acorn and pine seed in corrected estimates did not differ from experimental diets. Composition of grass in corrected diets was overestimated by 5% (P = 0.051). Forbs were underestimated by 4.2% (P = 0.013) and arthropods were underestimated by 5.4% (P = 0.004). Corrected diet estimates were better than fecal estimates for all diet items, except arthropods based on standard error estimates of deviations from known diet composition.

#### Discussion

Underestimation of mast items in diets of turkeys is predictable and within the theoretical considerations of

Table 1. Percent composition of acorn, pine seed, grass, forb, and arthropod in experimental diets, microscopic fecal estimates and corrected fecal estimates of turkeys.

	Acorn			Pine seed			Grass			Forbs			Arthropods		
	Diet	Fecal	Cor.	Diet	Fecal	Cor.	Diet	Fecal	Cor.	Diet	Fecal	Cor.	Diet	Fecal	Cor.
1	12.0	8.7	19.5	12.0	0.8	5.9	40.0	65.2	54.0	10.0	2.6	2.2	26.0	22.4	18.4
2	38.0	25.8	39.7	19.0	2.8	16.2	8.0	14.1	8.7	25.0	38.7	23.6	10.0	19.0	11.8
3	19.0	16.7	30.7	40.0	9.7	30.1	18.0	41.9	22.4	18.0	26.2	13.6	5.0	6.3	3.2
4	50.0	18.8	32.9	5.0	1.4	10.9	20.0	42.5	29.9	10.0	11.5	8.1	15.0	26.0	18.2
5	5.0	1.6	6.3	50.0	20.5	45.5	20.0	41.2	25.7	10.0	29.7	18.2	15.0	6.9	4.3
6	33.0	13.8	26.9	25.0	16.1	40.7	7.0	25.3	11.9	15.0	26.5	12.3	21.0	18.1	8.3
7	27.0	11.7	24.1	33.0	18.3	43.3	10.0	32.8	15.2	30.0	36.7	17.2	0.0	0.5	0.2
8	20.0	8.0	18.2	60.0	20.5	44.9	10.0	57.4	29.8	5.0	3.0	1.5	5.0	11.2	5.7
9	60.0	40.9	54.4	20.0	2.6	15.3	20.0	53.0	28.5	5.0	1.2	0.6	5.0	2.4	1.2
10	5.0	6.5	16.2	80.0	43.1	66.9	5.0	20.1	6.0	0.0	0.1	0.0	10.0	28.9	10.8
11	80.0	78.6	83.9	5.0	1.7	10.3	5.0	9.6	3.8	0.0	0.0	0.0	10.0	7.8	2.0
12	60.0	66.3	75.1	20.0	13.1	35.9	5.0	5.7	-3.6	5.0	1.2	-0.7	10.0	13.5	-6.7
13	15.0	6.0	15.7	70.0	70.7	87.1	5.0	12.8	-1.8	5.0	5.5	-0.2	5.0	5.0	-0.8
14	30.0	19.2	33.4	40.0	33.9	58.8	10.0	28.7	4.8	5.0	2.1	0.3	15.0	16.1	2.8
15	20.0	<u>6.7</u>	<u>16.6</u>	<u>10.0</u>	<u>3.3</u>	<u>17.5</u>	<u>25.0</u>	64.9	47.7	25.0	10.5	<u>7.6</u>	20.0	<u>14.6</u>	10.6
Mean <sup>1</sup>	31.6	21.9A	32.9	32.6	17.2A	35.3	13.9	34.3A	18.9A	11.2	13.0	7.0A	11.5	13.2	6.0A
SE <sup>2</sup>		13.4	8.0		19.8	11.4		24.0	10.1		8.7	7.0		7.0	8.1

<sup>&</sup>lt;sup>1</sup> Means followed by letter A differ from experimental diet  $P \le 0.05$  in paired t-test.

<sup>&</sup>lt;sup>2</sup> SE estimates deviations of corrected and fecal estimates from known diet composition per Reese 1960.

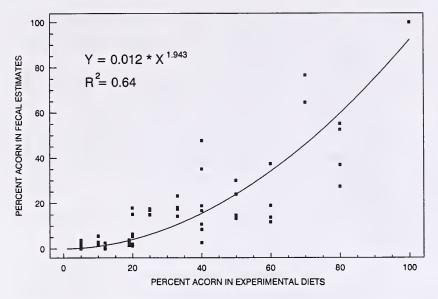


Figure 1.—Nonlinear regression of percent acorn composition in microscopic fecal estimates on percent acorn composition of experimental diets fed to turkeys.

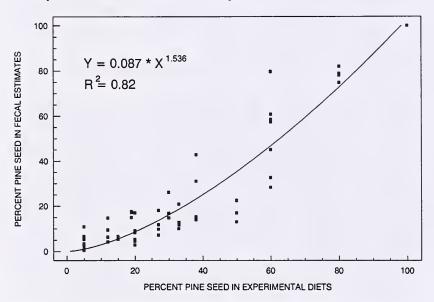


Figure 2.—Nonlinear regression of percent ponderosa pine seed composition in microscopic fecal estimates on percent ponderosa pine seed composition of experimental diets fed to turkeys.

the microscopic fecal technique for estimating diet composition. Several authors have suggested that correction factors should be applied to results of microscopic fecal estimates of diet composition to account for differential digestibility of plant materials (Dearden et al. 1975; Pulliam 1978). However, Vavra et al. (1978) found regression analyses are of little value in determining digestibility relationships of grasses and forbs in cattle diets. Holechek and Valdez (1985b) suggested that corrections for forage digestibility are unnecessary. The following factors probably obscure digestibility relationships in plants: high epidermal cell surface area; dry weight ratio in vegetative plant parts; technique variability; and technician error.

Because the bias in estimates of acorn or pine seed in diets declines as composition of either approaches 100% in a sample, the amount of underestimation available for other mast declines also. This interaction was apparent in two experimental diets (numbers 12 and 13). The result of this interaction, when one or both mast items comprise >70% of the diet, is that corrected estimates of items comprising a small portion of the diet may be <0. We recommend that if multiple mast items occur in diets and composition of one or both exceed 70% from microscopic fecal analysis, no correction should be applied.

In management situations, it probably is not possible to identify and manage minor species of food items—unless they contain some required nutrients or minerals not found elsewhere. Corrected estimates of diets clearly identified the predominant food items. Average composition was within 5% of experimental diets. Corrected diets provided less biased estimates of diet composition than uncorrected fecal estimates.

The relationships between diet composition and microscopic fecal estimates reported here are not intended to be applied beyond the scope of this study. Our data demonstrate that the theoretical basis for biases of estimates is inherent in the microscopic fecal technique if diets contain mast items. We recommend that researchers develop similar relationships to improve estimates of diet composition if hard mast items are expected to occur in diets of animals under investigation. The microscopic fecal technique for estimating diet composition of herbivorous animals can provide researchers with quantitative data of diets if: bias associated with mast items are accounted for; the technique is properly applied; and technicians are properly trained (Rogers and Uresk 1974; Johnson et al. 1983a; Alipayo et al. 1992; this study).

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Wild and domestic dark turkeys (Meleagris gallopavo) were fed experimental diets containing acorn (Quercus gambelli), ponderosa pine (Pinus ponderosa) seed, grasses, forbs, and arthropods. In fecal estimates of diet composition, acorn and ponderosa pine seed were underestimated and grass was overestimated. Regression of acorn and pine seed in experimental diets with microscopic fecal estimates indicated significant nonlinear relationships. Based on regression analyses, corrected acorn and pine seed composition in diets did not differ from experimental diets. Corrected estimates for grass were slightly overestimated. Corrected estimates for forbs and arthropods were slightly underestimated. Corrected diet estimates had smaller standard errors than microscopic fecal estimates.

**Keywords**: fecal analyses, microscopic technique, microhistological technique, turkey (*Meleagris gallopavo*) diets.

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